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Migration and brackish environment use of *Prochilodus lineatus* (Characiformes: Prochilodontidae) inferred by Sr:Ca ratio transects of otolith

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The streaked prochilod, *Prochilodus lineatus*, represents the most important fishery in the La Plata Basin (South America). Our objective was to analyze brackish environment use by the streaked prochilod captured from Paraná and Uruguay rivers. To accomplish this, *lapillus* otolith sections were analyzed for Sr:Ca with laser ablation-inductively coupled plasma-mass spectrometry (LA ICP-MS) to infer habitat use of fish. To the interpretation of transects, a threshold that represents the transition between freshwater and brackish environments was calculated using the Sr:Ca ratio of the otolith edge of specimens captured in the first section of the La Plata Estuary (salinity ≥ 0.5 PSU). The percentage of fish using the estuary was higher in the Paraná (37%) than the Uruguay River (5%). Change-point analysis showed that fish entered the estuary between 1 and 3 times throughout life at a wide range of ages (0-15 years). These incursions had no obvious periodicity. This information should be integrated into future management actions, which should also be specific to each area since migration patterns differ between the major rivers of the basin.

Keywords: Connectivity, Freshwater resident, Laser ablation, River migration, Streaked prochilod.

El sábalo, *Prochilodus lineatus*, representa la pesquería más importante en la Cuenca del Río de la Plata (Sudamérica). Nuestro objetivo fue analizar el uso del hábitat estuarino del sábalo proveniente de los ríos Uruguay y Paraná. Para esto, se analizó la relación Sr:Ca en secciones de otolitos *lapilli* por ablación láser acoplada a espectrometría de masas con fuente de plasma de acoplamiento inductivo (LA ICP-MS) para inferir el uso de hábitat. Para interpretar las transectas, un umbral que representa la transición entre los ambientes de agua dulce y estuarino, fue calculado usando la relación Sr:Ca del borde del otolito de especímenes capturados en la primera sección del estuario del Plata (salinidad ≥0.5 UPS). El porcentaje de peces que usaron el estuario fue más elevado para el Paraná (37%) en relación al Uruguay (5%). El análisis de cambio puntual mostro que los individuos ingresan al estuario entre 1 a 3 veces a lo largo de la vida en un amplio rango de edades (0-15 años). Las incursiones no mostraron una periodicidad notoria. Esta información debería integrarse a futuras acciones de manejo que deberían ser específicas para cada área considerando los patrones de migración que difieren entre los grandes ríos de la Cuenca.

Palabras clave: Ablación laser, Agua dulce residente, Conectividad, Migración de rio, Sábalo.

Introduction

The La Plata Basin, with 3,170,000 km^2 is the fluvial-marine system with larger surface of the Americas, after the Amazon. This basin goes through 5 countries in South America and is located between latitudes 17° S and 36° S, with a north-south current direction (Fig. 1). Its most important rivers are the Paraná (4,000 km long), Paraguay (2,600 km long), Uruguay (1,800 km long) and Pilcomayo (1,500 km long) (Fig. 1) (Guerrero *et al*., 1997). These rivers drain into the Paraná Delta, which terminates in the La Plata Estuary (Guerrero *et al*., 1997). The main fishery resource

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of the basin is the streaked prochilod, *Prochilodus lineatus* (Valenciennes, 1837), whose catch volumes have exceeded 36,000 t/year, from the middle portion of the Paraná River (Sverlij *et al*., 1993; MINAGRO, 2016). Streaked prochilod reproductive cycle is correlated with the natural flood pulse regime (Neiff, 1999) with migrations upstream and spawning in open river waters coupled to the flooding periods as a mechanism of dispersion of eggs (Sverlij *et al*., 1993). According to Bonetto *et al*. (1981), Delfino, Baigun (1985) and Espinach Ros *et al*. (1998), the streaked prochilods migrate over 1,000 km to feed and reproduce.

Considering that the La Plata estuary (Uruguay and Argentina) is one of the target sites for commercial fishing, life history information on streaked prochilod from brackish environments is needed to support management decisions about this resource. The studies of capture, tagging and recapture have provided interesting information about brackish incursions. However, these studies have not been able to determine the proportion of individuals using the estuary, and the ages at which brackish environment use occurred. For example, Espinach Ros *et al*. (1998) have reported displacements of up to 1,100 km for fish tagged in the Uruguay River and recaptured in the Paraná, as well as movements between Uruguay River and the La Plata Estuary. It has also been reported a tagged specimen in Buenos Aires City (near the limit of the estuary), which was recaptured 1,500 km upstream in the Paraná River (Sverlij *et al*., 1993). It seems that there is an important connectivity between groups of fish from Paraná and Uruguay rivers with the estuary.

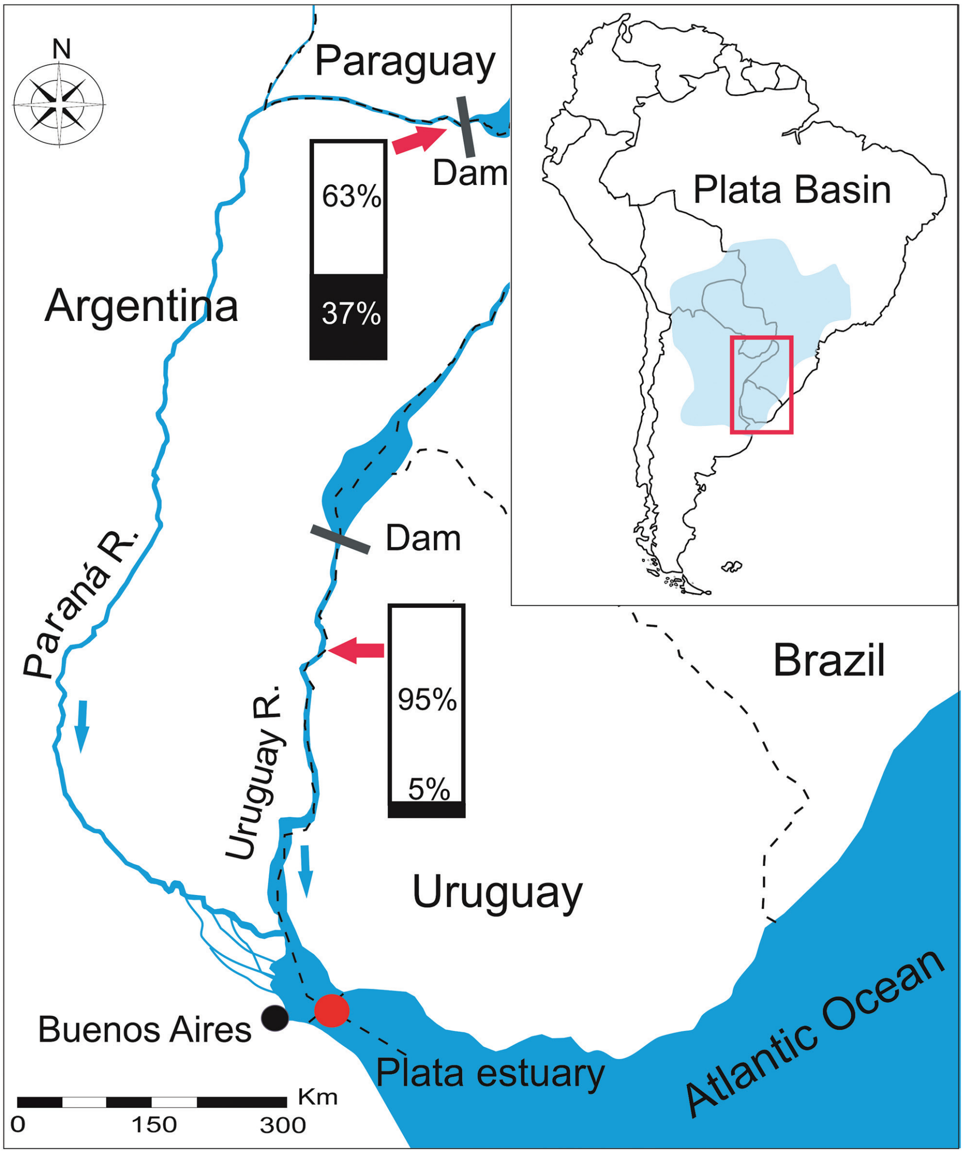

Fig. 1. Sampling sites of *Prochilodus lineatus* (red arrows). Red point shows the sampling site of fish use for transition threshold estimation between freshwater and brackish environments. The bar charts show the proportion of each migratory pattern for each collection site (black: freshwater straggler; white, freshwater resident).

Tagging studies result extremely expensive due to the extension of the basin and the number of specimens needed to be tagged to obtain a representative number of recaptures (Begg, Waldman, 1999). In the past two decades, the chemical composition of fish otoliths has been developed as a natural tracer of habitat use and cost-effective alternative to mark recapture investigations. Otoliths are structures composed mainly of calcium carbonate deposited as aragonite, vaterite or calcite crystals in a protein matrix (otoline) found in the inner ear (Campana *et al*., 1997). Chemical composition is preserved in the otolith chronology because deposited material is not reabsorbed or altered (Campana, Neilson, 1985; Casselman, 1990; Campana, Thorrold, 2001; Elsdon *et al*., 2008). Trace elements in combination with growth rings result in an important file that records environment information experienced by fish, environmental changes and exposure to pollutants throughout ontogeny (Halden, Friedrich, 2008). Because many cationic trace elements substitute for calcium in the otolith chemical matrix, element ratio, such as Sr:Ca, (rather than single element concentrations) provide the most accurate representation of chemical gradients in the water (Bath *et al*., 2000). In estuaries, salinity often has a positive correlation with otolith Sr:Ca when the freshwater end-member has lower Sr:Ca than sea-water (Secor, Rooker, 2000; Kraus, Secor, 2004; Martin *et al*., 2004; Sturrock *et al*., 2012). The techniques used to study ontogenetic changes of Sr:Ca ratio in otoliths include spot and line scan analysis by electron probe micro-analysis (EPMA), microproton-induced X-ray emission (micro-PIXE) (Lin *et al*., 2007; Hedger *et al*., 2008; Daros *et al*., 2016), and more recently laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) (Morales-Nin *et al*., 2014; Fowler *et al*., 2016; Kissinger *et al*., 2016; Avigliano *et al*., 2017b; Callicó Fortunato *et al*., 2017).

Considering the lack of knowledge about the use of environments with different salinities, our objective was to analyze brackish environment use by the streaked prochilod captured from the La Plata Basin (Paraná and Uruguay rivers). In particular, we evaluated the proportion of individuals using the estuary, and the ages at which brackish environment use occurred. We accomplished this by analyzing transects of Sr:Ca in *lapilli* sections using LA-ICP-MS.

Material and Methods

Sample collection. Fish were collected between February 2011 and November 2014 by using trammel nets in the Uruguay River, (31°25.246'S-58°1.407'W-31°59.105'S-58°9.599'W) (Corrientes and Entre Ríos provinces, Argentina-Uruguay international boundary) and in the Paraná River (27°25.467'S-58° 48.133'W) (Corrientes province, Argentina-Paraguay international boundary) (Fig. 1). Additionally, 9 adult fish were collected at the boundary between freshwater and the estuary (34° 41.969'S- 57°40.291'W) (inner parts of the La Plata Estuary, salinity ≥ 0.5 PSU).

Once the nets were recovered, the fish were killed with percussive stunning (Van De Vis *et al*., 2003). Fish were placed on ice and transported to the laboratory where they were measured (standard length=SL) and the *lapilli* otoliths were extracted. We preferred using *lapilli* otoliths rather than *sagittae* or *asterisci* otoliths because they were larger and allowed less measurement error (Assis, 2005; Avigliano *et al*., 2015a).

Otoliths from Uruguay River and La Plata estuary were deposited in the collection of the Continental Fishing Laboratory of the Ministry of Agro-industry from Buenos Aires, Argentina (vouchers numbers: 124579-131140) while Paraná River otoliths were deposited in the National University of the Northeast, Corrientes, Argentina (vouchers numbers: 2011/1-2011/223, 2013/7-2013318, 2014/3-2014/33).

Age determination and otolith preparation. Otoliths were weighed using a Sartorius AG ED 2242 (Göttingen, Germany) analytical balance, washed with Milli-Q water with a resistivity of 18.2 mΩ/cm and dried. The left otolith of each pair was embedded in epoxy resin and sectioned transversely through the core to a thickness of 700 µm using a Buehler Isomet low speed saw (Hong Kong, China) equipped with twin diamond edge blades and spacers. The number of *annuli* in the otolith section was counted with the piece immersed in ultrapure water, using a stereomicroscope (Leica EZ4-HD, Singapore) at 40X magnification. Age estimation by counting the *annuli* in *lapilli* otoliths of *P. lineatus* was validated by Espinach Ros *et al*. (2008). To avoid age effect on otolith chemistry, only fish between 12-15 years were selected for analysis (21 for Uruguay River and 30 for Paraná River) (Tab. 1).

Tab. 1. Descriptive statistics of individuals from each sampling site. N: sample size; SD: standard deviation.

	Age (year)			Standard length (cm)			N
	mean±SD		range	mean±SD		range	
Uruguay River	13.8	0.9	12-15	46.3	3.4	46-54	21
Paraná River	14.0	1.3	12-15	43.8	5.6	36-58	30

Otolith sections were fixed to glass slides using clear epoxy resin. In addition, the potential exists of vateritic inclusions was evaluated by observed under reflected light after EDTA etching (Tzeng *et al*., 2007). Otolith sections were rinsing 3 times in Milli-Q water and drying in a laminar flow hood.

Determination of Sr:Ca ratio by LA-ICP-MS analysis. Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) analyses were conducted at University of Oviedo, Spain, using a 193nm ArF* Excimer laser ablation system (Photon Machines Analyte G2) coupled to an ICP-MS Agilent 7700 (Agilent Technologies, Santa Clara, USA). Analytical conditions of the LA-ICP-MS system are summarized in Tab. 2.

Tab. 2. LA-ICPMS operating conditions.

Instrument	Parameter	Value
ArF 193nm laser ablation		
	Laser fluence	12 J/cm^2
	Repetition rate	10 Hz
	Pitsize diameter	30 µm
	Ablation Cell	Two-volume HelEx cell
	Cell gas Flow	He - 0.8 l/min
	Scan Speed	5µm/s
ICP-QMS		
	Acquisition mode	Time resolved
	Nebulizer gas flow	Ar - 0.9 l/min
	Isotopes measured	^{43}Ca and ^{88}Sr
	Integration time	210 ms/isotope

Helium was used as the carrier gas in the ablation cell and Ar was added before entering the ICP. The ion optics were adjusted to yield maximum sensitivity and balanced mass response while ablating National Institute of Standards and Technology standard reference material SRM NIST 612 glass. The optimization was carried out manually while monitoring $^{7}Li^{+}$, $^{133}Cs^{+}$, $^{232}Th^{+}$, $^{238}U^{+}$ and $^{232}Th^{16}O^{+}$ ion signal intensities. Plasma robustness was monitored via the $^{232}Th^{16}O/^{232}Th$ and the $^{238}U/^{232}Th$ intensity ratios. ThO^{+}/Th^{+} intensity ratios were always below 0.4% and $^{238}U^{+}/^{232}Th^{+}$ intensity ratio was less than 1.2. Additionally, the cross calibration of the pulse and analogue stages of the scanning electron microscope detector ("PA-factor") was carried out daily to ensure a linear response of the instrument of >8 orders of magnitude for the isotopes of interest.

NIST SRM 612 silicate glass reference material was employed as an external reference material (Pearce *et al*., 1997; Jochum *et al*., 2011; NIST, 2012) to quantify the concentration of Sr, using a fixed value for Ca as an internal standard. Calcium concentration of the otolith matrix was assumed to be 38.8 wt.% (Yoshinaga *et al*., 2000 ; Hamer *et al*., 2015). In particular, ion signals from $^{43}Ca^{+}$ and $^{88}Sr^{+}$ were measured in the otoliths and in NIST 612, using the laser ablation scan mode (transects). Ion signals were collected before the ablation process to determine their background level and during the ablation process. Net ion signals were employed in the bracketing quantification method, where the reference material (NIST 612) is analysed at the beginning and at the end of each analysis session to monitor and correct for any signal drift. The maximum deviation observed during one analytical session was about 5%. For instance, the average concentration of Sr measured in NIST 612 during the analytical session was 78 ± 4 ppm, which is in good agreement with the nominal value of 78.4 ± 0.2 ppm (NIST, 2012). The limit of detection (LOD) for Sr was 0.0023 mmol/mol, calculated using the 3 sigma criteria. Concentration of Sr was expressed as molar ratios (element:Ca = mmol/mol) to account for fluctuations in the amount of material analyzed and the loss of material during the preparation process (Sinclair *et al*., 1998; Bailey *et al*., 2015).

Pattern classification and data analysis. Similar to Albuquerque *et al*. (2010), Sr:Ca ratio of the otolith edge (last spot of the transect) was compared among sampling sites using Kruskal Wallis test, in order to evaluate whether there is coherence with the capture sites (Uruguay and Paraná Rivers and La Plata Estuary) and the salinity associated with them (a higher Sr:Ca ratio is expected near the estuary). Data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests respectively. Prior to Kruskal Wallis test, we evaluated the association between the Sr:Ca ratio of the otolith edge and the fish age using Spearman correlation. The existence of a correlation with the age could affect the interpretation of the Sr:Ca ratio.

To facilitate the interpretation of transects, the threshold that represents the transition between the freshwater environment and the estuary was calculated using the Sr:Ca ratio of the edge of the otolith (last spot of the transect) of 9 additional specimens captured in the first section of the La Plata estuary (salinity $\geq$ 0.5 PSU) (Fig. 1). The threshold was estimated as the average of Sr:Ca ratio of the otolith edge plus twice the standard deviation (mean+2* SD) as suggested by Lin *et al*. (2015) and Avigliano *et al*. (2017b).

Several algorithms have been used to facilitate the interpretation of fish movement patterns and to quantify the number of habitat changes during their life history (Hedger *et al*., 2008; Walther *et al*., 2011; Killick, Eckley, 2014; Freshwater *et al*., 2015; Hegg *et al*., 2015; Wynne *et al*., 2015). In this work we used Change-Point analysis (CPA) to facilitate the classification of individuals and quantify the number of changes in otolith elemental ratio (Shrimpton *et al*., 2014; Hegg *et al*., 2015). We assumed that significant changes in the transect corresponded with habitat changes due to chemical composition of the water (Freshwater *et al*., 2015; Hegg *et al*., 2015; Wynne *et al*., 2015). CPA determined whether there had been a change in the underlying process that generated the sequence of events and identified where the change occurred. The procedure used to perform a CPA comprises a combination of cumulative sum charts and boot strapping to identify shifts in the pattern of Sr:Ca. The analysis provides both confidence levels and confidence intervals for each change (95% confidence is used for all confidence intervals) and it is robust to issues of non-normality (Shrimpton *et al*., 2014). The Change-Point Analyzer 2.3 software package (Taylor, 2000) was used for CPA.

Transects, thresholds and change points in the Sr:Ca chronologies were graphically inspected to infer movement between freshwater and brackish environments (Wynne *et al*., 2015).

In this work, patterns were defined based on habitat use assuming an approach similar to that of Elliott *et al*. (2007) as follows (Fig. 2): 1) freshwater resident: defined as permanence in freshwater throughout life and 2) freshwater straggler: defined as freshwater fish found in estuaries in low number and whose distribution is usually limited to the low salinity in upper reaches of estuaries.

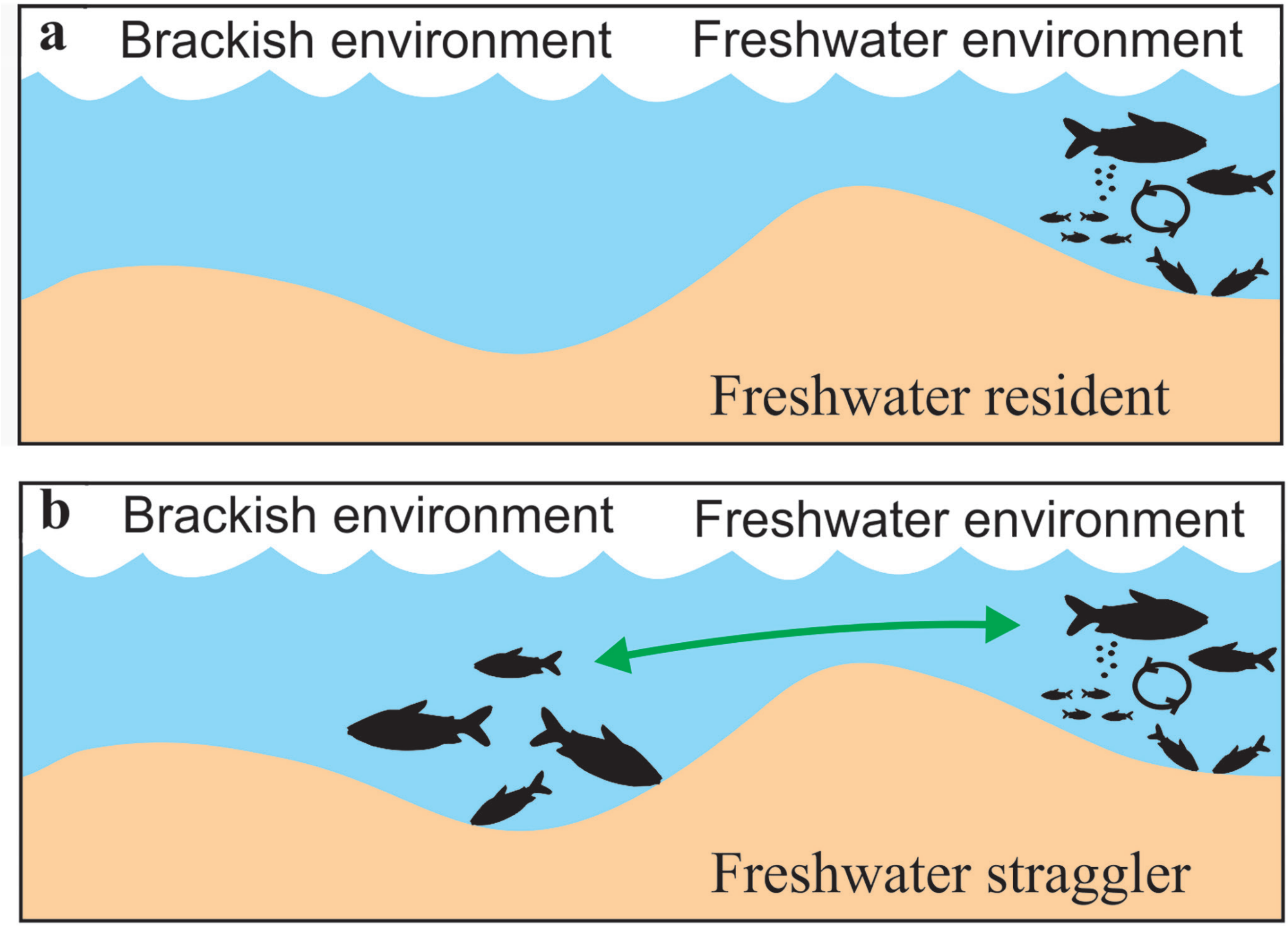

Fig. 2. Habitat use patters according to Elliott *et al*. (2007). **a.** freshwater resident and **b.** freshwater straggler. Modified from the fig. 1 of Potter *et al.* (2015).

Following Walther *et al*. (2011) and Shrimpton *et al*. (2014), the variable "number of changes" was compared between locations (Uruguay and Paraná rivers) using the non-parametric Mann-Whitney U test because the response was not normally distributed with heterogeneous variance (Shapiro-Wilk, $p < 0.05$; Levene, $p < 0.05$), even after transformation log (x+1). Prior to parametric Mann-Whitney U test, we evaluated the association between the number of Sr:Ca changes and the fish age using Spearman correlation.

Results

Otolith edge and threshold estimation. Otolith edge had Sr:Ca values of 1.19±0.22, 1.33±0.33 and 1.66±0.22 mmol/mol for the Uruguay and Paraná Rivers and Estuary, respectively (Fig. 3), being significantly higher for the latter ($H=13.3$, $p<0.009$). No correlation was found between age and Sr:Ca ($r=-0.24$, $p=0.1$). For this reason, it was not necessary to make age corrections in variables.

A reference value for movements between freshwater and brackish environments was calculated as the average Sr:Ca value + 2*SD (1.66 mmol/mol + 2*0.22=2.12 mmol/mol) at the otolith edge of 9 specimens caught in the first section of the estuary (Fig. 1). Values greater than the Sr:Ca=2.12 mmol/mol threshold were considered indicative of brackish environment use.

Otolith microchemical profiles of specimens caught in the estuary are shown in the complementary figure **S1** - Available only as online supplementary file accessed with the online version of the article at http://www.scielo.br/ni.

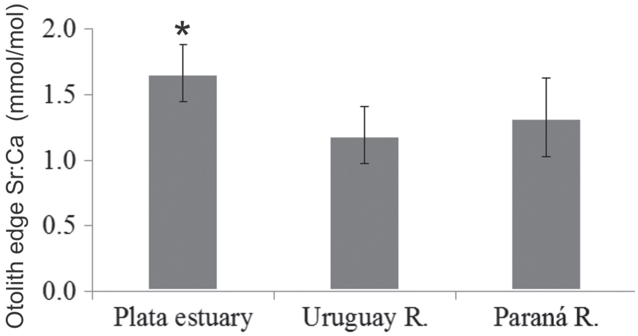

Fig. 3. Otolith edge Sr:Ca ratio of *Prochilodus lineatus* calculated for freshwater and brackish environments. Asterisks indicate a statistically significant ($p<0.05$) difference between sampling sites.

Life history and migration patterns allocation. Considering the fish captured in fresh water, the otolith Sr:Ca ratio ranged between 0.34 and 3.19 mmol/mol (mean ± SD: 1.33 ± 0.33 mmol/mol). Fish had values of Sr:Ca in the otolith core and edge that were less than the reference value, suggesting that these were hatched and caught in freshwater environments (Fig. 4).

The percentage of each migratory behavior is represented as a percentage in bar charts for each sampling site (Fig. 1). Freshwater resident pattern was observed in 95% (N=20) and 63% (N=19) while, freshwater straggler was found 5% (N=1) and 37% (N=11) in Uruguay and Paraná Rivers, respectively (Fig. 1).

According to the results of the CPA and the estimated threshold, clear patterns were not observed in relation to the ages at which the brackish incursions occur (Fig. 4). Of all fish classified as freshwater straggler, 46.4% were predicted to make brackish incursions between age-3 and age-5. Only 30.6% of freshwater straggler specimens were predicted to enter the estuary more than once.

Specifically, the only freshwater straggler specimen captured in the Uruguay River performed only one brackish incursion at the age of 5 (Fig. 4f).

Among the 11 freshwater straggler fish caught in the Paraná, 4 performed a single incursion between the 3rd and the 4th year of life. Three other specimens performed a single incursion at the ages of 1, 8 and 14 years respectively. Finally, 4 specimens performed between 3 and 4 incursions at the ages of 12 and 13; 1, 2 and 4; 0^{+}, 7 and 11; 2, 10-12 and 15 years, respectively (Fig. 4b-c).

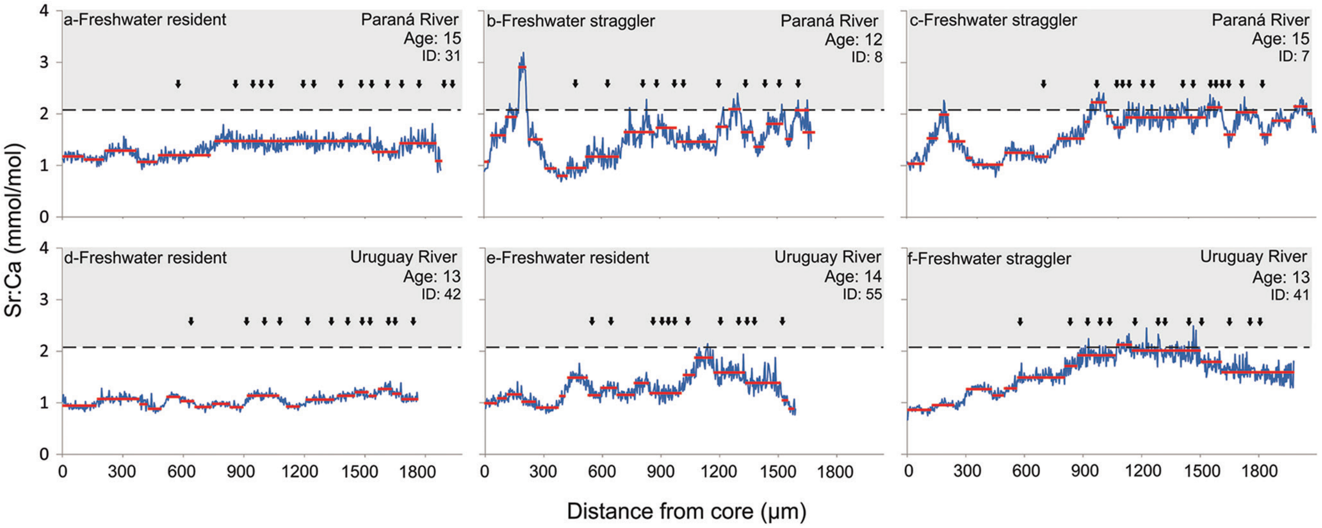

Fig. 4. Otolith microchemical profiles of *Prochilodus lineatus* from core to edge (age: 12-15). Vertical arrows indicate otolith *annuli* (age) and black dashed line indicate upper (brackish) and lower (freshwater) thresholds for Sr:Ca. Horizontal lines illustrate stable signatures identified by using change-point analysis for the Sr:Ca ratio.

Quantification of changes in the life history. The global average (mean ± SD) for the number of changes of environment (or change points per transect) was 20.5 ± 5.4 (range: 9-33). Number of changes in transects of Sr:Ca were 18.5 ± 5.3 and 22.5 ± 5.6 for Uruguay and Paraná Rivers, respectively.

Spearman test showed that there is no correlation between age and number of changes points (r=-0.25, p=0.08). The Mann-Whitney U test showed significant differences in the number of changes in transects of Sr:Ca between sampling sites (U=810, p=0.0001), being higher in the Paraná River.

Discussion

While transition thresholds between environments seem to vary among teleost fish, our threshold estimate for brackish environment use (Sr:Ca=2.12 mmol/mol) was comparable to benchmarks for other species. For example, Bradbury *et al*. (2008) has reported values ~2.3 mmol/mol for *Osmerus mordax* (Mitchill, 1814) while Smith, Kwak (2014) reported values of ~3 mmol/mol for *Gobiomorus dormitor* Lacepède, 1800. However, euryhaline species were associated with significantly higher values of reference, as *Mugil cephalus* Linnaeus, 1758 (Sr:Ca=3.3 mmol/mol) (Fowler *et al*., 2016), *Genidens barbus* (Lacepède, 1803) (Sr:Ca=3.75 mmol/mol) (Avigliano *et al*., 2017b) and *Zenarchopterus dunckeri* Mohr, 1926 (Sr:Ca=4.2 mmol/mol for freshwater-brackish) (Kanai *et al*., 2014).

Low La Plata Basin has a salinity gradient in north-south direction, which is positively correlated with Sr:Ca ratio of water (Avigliano, Volpedo, 2013). For this reason, the Sr:Ca ratio of otolith has been widely used to infer migratory and population aspects of several species (Avigliano, Volpedo, 2016); for example *Genidens barbus* (Avigliano *et al*., 2015a,b, 2016a, 2017b), *Odontesthes bonariensis* (Valenciennes, 1835) (Avigliano, Volpedo, 2013; Avigliano *et al*., 2014, 2015c), *Mugil liza* Valenciennes, 1836

(Callicó Fortunato *et al*., 2017), *Lycengraulis grossidens* (Spix & Agassiz, 1829) (Mai *et al*., 2014), *Micropogonias furnieri* (Desmarest, 1823) (Albuquerque *et al*., 2012) and *P. lineatus* (Avigliano *et al*., 2016b, 2017a). Particularly in *P. lineatus* it has been used the otolith microchemistry (Sr:Ca, Ba:Ca and Zn:Ca ratios) as well as otolith and scales morphometry in order to identify different nursery areas (Avigliano *et al*., 2016b, 2017a). According to Avigliano *et al*. (2017) the capture sites studied in this work are also different nursery areas. In contrast to previous publications on the species, this paper describes for the first time details on brackish use, revealing that a considerable proportion of fish from Paraná River, depending on the sampling site, uses this environment throughout his life.

Other methodologies have been used to study migratory and biological aspects of the streaked prochilod, primarily tagging and recapture (Bonetto et al., 1981; Delfino, Baigun, 1985; Sverlij et al., 1993; Espinach Ros et al., 1998) although biochemical methods (Colombo *et al*., 2011; Speranza *et al*., 2012) and distribution studies (Bayley, 1973; Stassen *et al*., 2012) have also been conducted. These studies show brackish use in some specimens, but also suggested the presence of non-migratory fish, especially in the Uruguay River (Bonetto *et al*., 1981; Delfino, Baigun, 1985; Espinach Ros *et al*., 1998). Our results provide further evidence of this connectivity and revealed that streaked prolichod in the estuary utilize this habitat intermittently throughout their lives. Espinach Ros *et al*. (1998) have also reported the existence of adult specimens that did not migrate (evaluated period of 3 years) with respect to the place tagged on the Uruguay River, suggesting the existence of resident populations. In this work, the highest percentage of freshwater resident specimens was registered in the Uruguay River. However, it is not possible to affirm that these specimens are not migratory, as they could move long distances among environments with similar salinity and make no significant differences in Sr:Ca ratio of otolith (Avigliano *et al*., 2017a).

According to CPA, the greatest number of changes was observed for the Paraná River, which is consistent with the highest percentage of freshwater stragglers specimens (Fig. 1).

It is clear that the use of environments with different salinities is complex and highly variable between sites and specimens. In this sense, the variability in the observed migration patterns could be associated with different evolutionary adaptations (Lucas, Baras, 2001; McDowall, 2001; Elliott *et al*., 2007). Brackish incursions could also facilitate connectivity between the great rivers. This way fish could move into new environments and perhaps into more beneficial ones in terms of food and reproduction, especially if the natal site had changed.

Our results are important for the management of the shad resource. Even though there are bi-national organisms that monitor the resource (Comisión Administradora del Río Uruguay, CARU), there is no management of it at a basin or sub-basin level. Even integral management is not carried out within some countries. For example, in Argentina each province has independent regulations and handle closed seasons, minimum catch sizes and different fishing quotas. Even Buenos Aires province (Argentina) has banned the fishing and commercialization of shad caught in the Río de la Plata since 2000 (SAP, 2000) due to the presence of contaminants found in muscle and in the estuary. It has been suggested that shad may transport dozens of tons of contaminants per year from the estuary to the Paraná and Uruguay rivers (Colombo *et al*., 2011). The results of the present study allow inferring that a proportion of the shads captured in the middle portion of the Uruguay and Paraná rivers use the estuary in different stages of its life. In this sense, the possibility of implementing regulations at the regional level, rather than local, should be discussed. Considering the use of brackish waters and migration distances, other methods of assessment and management should be considered in order to ensure not only the sustainability of the fishery but also the quality of the product for consumption. Management methods should consider connectivity between different points in the basin, paying special attention to brackish use by the species, since this could affect the quality of the meat and affect consumer health (Colombo *et al*., 2011; Speranza *et al*., 2012).

In conclusion, although it is known that streaked prochilod performs upstream migrations in winter for reproduction and downstream migration in summer to feed (Sverlij *et al*., 1993), occupation of brackish environments is highly variable among individuals with unknown consequences at the population level. Excessive commercial fishing may have rapid negative effects on resident populations. In this sense, populations should be managed integrally. Here, we have to emphasize the need to link the obtained results with current resource management actions in order to contribute to their sustainability. We highlight the importance of intensifying this type of studies including other markers such as stable isotopes of Sr, which would allow to track the movements of the specimens in freshwater throughout the basin (Hegg *et al*., 2015).

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